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Frequency and Spacing in the Circulus Formation of Scales in the Goldfish, *Carassius auratus*: Consequence of Temperature and Rations

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Abstract

Using the goldfish, *Carassius auratus*, frequency and spacing in the circulus formation of scales were examined in relation to changes in temperature and rations. Scales showed temperature-dependent growth rates and it took 6.8, 3.2, and 2.2 days for the formation of a single circulus in the groups kept at 16, 22, and 28°C, respectively. However, circulus spacing showed no difference between the 3 groups. Fish fed with a ration of 4% of body weight every other day required 5-7 days for the formation of a single circulus, compared with 2-3 days in fish fed with a ration of 4% or 8% every day. Circulus spacing widened in the latter groups. These results suggest that temperature and rations differently affect the circulus formation of scales, especially circulus spacing.

Introduction

Generally fish scales have well-defined circuli oriented at regular intervals. The disturbance of these circuli has been used as an annulus to age fish. If the frequency of circulus formation is regularly related with a chronological event, the number of circuli will provide us with various information necessary for reconstructing the life history of an individual fish. The width of circulus intervals (spacing) will also give us precious information on the rate of body growth in the past, if it is correlated with its somatic growth.

Temperature has been considered to be the main factor affecting circulus spacing (Krogius, 1958; Hopson, 1965; Bilton and Messinger, 1975), because it profoundly affects somatic growth. However, Ouchi (1969) examined the growth rate of scales in relation to temperature and showed that circulus spacing did not necessarily reflect the growth rate of scales in goldfish. Barber and Walker (1988) found that temperature was not the primary cause of spacing and hypothesized that the pattern of circulus spacing was affected by the photoperiod and food availability in sockeye salmon. Bilton and Robins (1971) also reported that circulus spacing was sensitive to small changes in feeding in the same species, while Bilton (1974) reported inconsistent results in circulus spacing between starvation and feeding periods. In spite of these studies, it remains to be studied what regularity is present in the frequency of circulus formation and its spacing in relation to scale and somatic growth.

Using goldfish, the present study was undertaken to provide more insight into

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the circulus formation of scales in relation to somatic growth controlled by changing temperatures or rations.

Materials and Methods

Goldfish (*Carassius auratus*) were obtained from a commercial dealer and acclimated to experimental conditions for at least 2 weeks before use. They were fed carp food pellets once a day during the acclimation and experimental periods.

Temperature experiment

Twenty-one fish weighing about 10 g were used for this experiment. After measuring body weight and standard length, they were given a single intraperitoneal injection of tetracycline (Takeda Chem. Ind.) at a dose of 0.1 mg/g body weight for time marking. Then they were randomly divided into 3 groups (7 each) and transferred to 3 aerated tanks (30 l each) for which the temperature was controlled at 16, 22, and 28°C. They were fed to the extent of no left-over food and were subjected to a sampling of scales and the measurement of body growth after 1 and 3 months. This experiment was conducted from June to September 1992.

Ration experiment

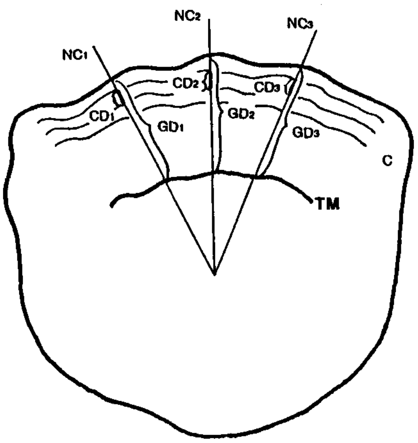
Thirty fish weighing about 13 g were measured for standard length and body weight, and given a single injection of tetracycline as described above. Then they were divided into 3 groups (10 each) and transferred to separate tanks (50 l) containing aerated tap water maintained at 25 ± 1°C. Two groups were given ration sizes of 8% and 4% of body weight every day and the other group was given a ration of 4% every other day. They were designated as high, medium and low ration groups, respectively, and sampled for examination of scale and body growth after 1 and 3 months. This experiment was carried out from October 1992 to January 1993.

Scale sampling and preparation

Two scales were removed with forceps from a defined area in the dorsoanterial trunk of each fish. Special attention was paid not to include regenerating scales. Scale sampling was alternately made from the left and right sides of the trunk after 1 and 3 months, respectively. Scales were rinsed in distilled water, rightly plotted with paper to remove mucous and epidermis, and mounted with 50% non-fluorescent glycerin on a microscopic glass slide for examination of tetracycline-induced fluorescence.

Measurement of scale growth parameters

The distance of accretion growth during the experiments was measured under ultraviolet illumination (Zeizz MPM) using an image analyser system (Image Command 4198). The distance between 2 adjacent circuli was also measured with this system. The number of circuli formed during the experiments was counted under an ultraviolet microscope (Nikon, V-16A). All the measurements were made on 3 axes of each scale (Fig. 1) and the mean values were used for analyses.



$$GD\ (\mu m) = \frac{GD_1+GD_2+GD_3}{3}$$

$$CD\ (\mu m) = \frac{CD_1+CD_2+CD_3}{3}$$

$$NC = \frac{NC_1+NC_2+NC_3}{3}$$

Fig. 1. Measurement of scale growth (GD), circulus distance (CD), and the number of circuli (NC) formed during the experiments. C: Circulus; TM: Tetracycline mark (after Ouchi, 1969).

Statistical analysis

One way ANOVA was employed for statistical analysis of data. Difference was considered significant when $P<0.05$.

Results

Temperature experiment

Experimental fish showed different rates of growth depending on the temperatures they were exposed to. Somatic and scale growth were obviously accelerated

Table 1. Rates of somatic and scale growth in goldfish kept at 3 different temperatures.

	Temperature (°C)	Body weight (mg/day)	Standard length (μm/day)	Scale growth (μm/day)
	16	165±3.2*	128±2.6	7.8±1.6
One month	22	225±2.4	264±4.1	14.4±0.3
	28	326±4.5	409±4.6	23.8±2.5
	16	145±2.1	176±2.9	5.7±0.6
Three months	22	169±1.9	205±4.2	8.6±0.7
	28	324±4.5	336±3.9	17.8±2.4

* Mean±SE for 6 or 7 fish. Values are significantly ($P<0.05$) different among 3 groups kept at different temperatures at any examination time.

Table 2. Number of days required for the formation of a single circulus in scales of goldfish kept at 3 different temperatures.

Temperature (°C)	Number of days	
	One month	Three months
16	6.6±1.0*	7.0±0.8
22	3.0±0.2	4.1±0.4
28	1.9±0.2	2.5±0.4

* Mean±SE for 6 or 7 fish. Values are significantly ($P<0.05$) different among 3 groups kept at different temperatures at any examination time.

as temperature increased (Table 1), indicating the isometric relationship ($r=0.83$–0.94) between these growth. However, growth rates were much higher during the first 1 month than the whole period (3 months) of experiment.

The frequency of circulus formation was also positively temperature-dependent (Table 2). In terms of mean values of all data, scale circuli were formed at rates of 1 per 6.8, 3.6, and 2.2 days in 16, 22, and 28°C, respectively. No chronological relationship was found between the number of days elapsed and the number of circuli formed.

The distance between adjoining circuli is presented in Table 3. Although it varied from 35 μm to 40 μm on the average, no statistical difference occurred among these 3 groups reared in different temperatures at any examination time. If these data were present in terms of the relationship between scale growth and circulus spacing, a low correlation coefficient of 0.23 was obtained in the temperature experiment (Fig. 2). Consequently, temperature (or temperature-induced growth rate) had no effect on circulus spacing in goldfish scales.

Ration experiment

Somatic and scale growth were correlated with the rations given (Table 4). A large difference was found between the group with the low ration and the other 2 ration groups. However, no statistical dfference in somatic and scale growth occurred between the groups given the medium and high rations, possibly because the medium ration almost appeased the feeding demand of fish.

Table 3. Distance between adjacent circuli in scales in goldfish kept at 3 different temperatures.

Temperature (°C)	Distance (μm)	
	One month	Three months
16	35.2±2.8*	36.9±2.3
22	39.4±4.3	37.0±1.3
28	39.8±2.1	38.0±1.9

* Mean±SE for 6 or 7 fish.

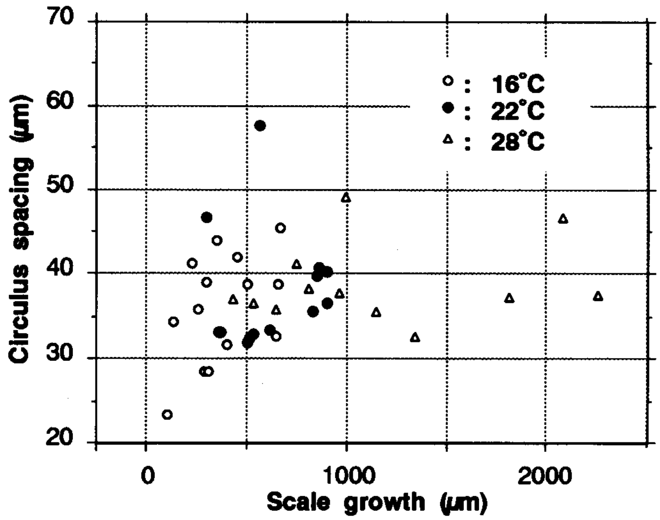


Fig. 2. Relationship between scale growth and circulus spacing in goldfish kept at 3 different temperatures.

Table 4. Rates of somatic and scale growth in goldfish fed on 3 different rations.

	Ration	Body weight (mg/day)	Standard length (μm/day)	Scale growth (μm/day)
One month	Low	91±1.6*	108±3.0	3.4±0.1
	Medium	355±2.4	315±2.7	15.5±1.0
	High	372±3.5	346±2.6	15.6±0.8
Three months	Low	132±1.5	158±1.2	7.5±0.9
	Medium	300±3.2	258±2.1	13.2±1.1
	High	314±2.3	302±1.6	16.0±0.8

* Mean±SE for 7-10 fish. Values are significantly ($P<0.05$) different between the group with the low ration and the other ration groups at any examination time.

The low ration depressed the frequency of circulus formation, taking 5-7 days for the formation of a single circulus, compared with 2-3 days in the other groups (Table 5). No significant difference in the frequency was found between the medium- and high-rationed groups. These results show that scale circuli are formed depending on the ration-induced rate of growth.

It is characteristic that circulus spacing changed according to the growth rate induced by rations (Table 6). The average distance between adjacent circuli was 37.1, 44.8, and 43.6 μm in the low-, medium- and high-rationed groups, respectively. The spacing between circuli was significantly ($P<0.05$) narrower in the low-rationed group than that of the other groups after 1 month. However, a significant difference occurred only between the low- and high-rationed groups after 3 months.

Table 5. Number of days required for the formation of a single circulus in scales in goldfish fed on 3 different rations.

Ration	Number of days	
	One month	Three months
Low	7.2±1.5*	5.0±0.6
Medium	2.8±0.2	3.4±0.5
High	2.6±0.1	2.7±0.1

* Mean±SE for 7-10 fish. Values are significantly ($P<0.05$) different between the group with the low ration and the other ration groups at any examination time.

Table 6. Distance between adjacent circuli in scales in goldfish fed on 3 different rations.

Ration	Distance (μm)	
	One month	Three months
Low	35.1±1.8*	39.0±1.2
Medium	47.3±1.9	42.3±2.8
High	43.2±1.0	44.0±1.5

* Mean±SE for 7-10 fish. Values are significantly ($P<0.05$) different between one month's groups with the low and the other rations and between 3 months' groups with the low and high rations.

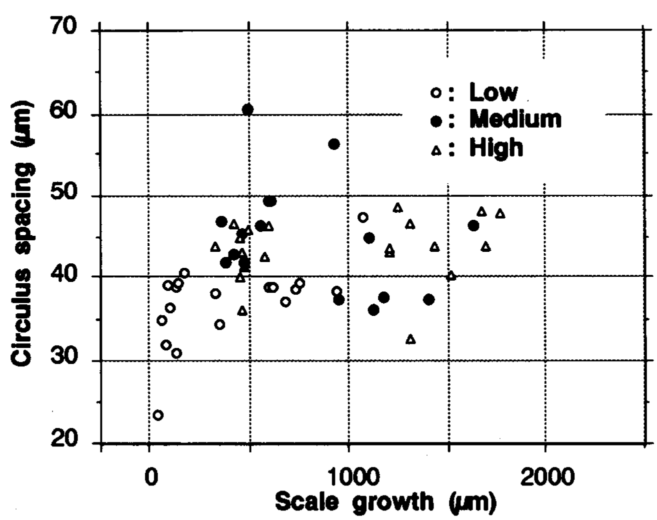


Fig. 3. Relationship between scale growth and circulus spacing in goldfish fed on 3 different rations.

Using data from all individuals in the ration experiment, a correlation coefficient of 0.31 was found between scale growth and circulus spacing (Fig. 3).

Discussion

Generally fish scales show isometric growth in relation to somatic growth. Such a relationship was seen in the present experiments. The frequency of circulus formation also positively related with the growth rate of scales in the temperature and ration experiments. However, it should be noted that the results of spacing were different between the two experiments. In the temperature experiment, circulus spacing was not affected by the rate of scale growth, while the spacing widened in the fish fed with sufficient rations, compared to the fish with the insufficient ration. These temperature-independent results on circulus spacing coincide with the results by Bhatia (1932) and Ouchi (1969) who reported that the fast or slow growth induced by changing environmental temperature had no effect on circulus spacing in rainbow trout and goldfish, respectively. Barber and Walker (1988) also reported that temperature was not the primary cause of circulus spacing in sockeye salmon. Accordingly, it seems reasonable to think that the temperature-induced fast growth of scales results in an increase in the frequency of circulus deposition without a change in spacing.

Rations had dual effects on circulus formation, affecting the frequency of circulus formation and circulus spacing in goldfish scales. Insufficiently fed fish showed a spacing of about 37 μm which was almost the same width as that of fish in the temperature experiment. This suggests that this value is the level of minimum spacing in this fish. Therefore significant difference in circulus spacing between the insufficiently and sufficiently fed groups is due to increased spacing in the latter groups. Similarly, Ikeda et al. (1974) reported no difference in circulus spacing between insufficiently fed and control groups in goldfish. Rations may exert an effect on circulus spacing not by reducing but by increasing the distance between circuli.

The present experiment showed no difference in scale growth, frequency of circulus formation, and circulus spacing between the 2 groups *i.e.*, the medium and high ration groups. These negative results are possibly due to the fact that the medium ration was sufficient to satisfy the appetite of the fish.

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